

Applicant : Gunnar Norstedt et al.
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Attorney's Docket No.: 13425-128001 / PH-1521-US

Amendments to the Specification:

Replace the paragraph beginning at page 2, line 33, with the following amended paragraph:

The ~~50~~ ~~52~~ bp SPI-GHRE SEQ ID NO:1; (GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG) was used to identify a core GH regulated sequence using gel electrophoresis mobility shift assay (GEMSA). Nuclear extracts were prepared and incubated with a ³²P labelled ~~50~~ ~~52~~ bp SPI-GHRE. Subsequently the extracts were analysed on polyacrylamide gels. The results showed that nuclear proteins, dependent on GH, bound to this DNA sequence. By competition with shorter oligonucleotides derived from SPI-GHRE a core GH sequence was identified. Based on certain sequence homologies to interferon response-elements we called this sequence SPI-GAS and also demonstrated that SPI-GAS functions as a GH regulated DNA element when put into a reporter vector. The core SPI-GAS has the following sequence; TTCTGAGAA.

Replace the paragraph beginning at page 3, line 7, with the following amended paragraph:

An expression plasmid containing a recombinant hormone responsive reporter consisting of six repeats of a ~~50~~ ~~52~~ bp growth hormone responsive element (GH-RE) from the serine protease inhibitor (SPI) 2.1 promoter fused to the thymidine kinase (TK) promoter was constructed. Corresponding constructs were made using the SPI-GAS element. Variants expressing either the bacterial protein chloramphenicol acetyl transferase (CAT) or firefly luciferase (SPI-CAT or SPI-Luc respectively) cDNAs were then constructed. Techniques to make these vectors are well known to experts in the field. The plasmid DNA constructions were transfected, together with plasmid expression vectors encoding either rat growth hormone receptors or mouse prolactin receptors, into Chinese hamster ovary (CHO), COS, and Buffalo rat liver (BRL) cells, using DOTAP liposomes and according to the manufacturer instructions. Cells were incubated overnight with DNA and DOTAP in serum free media, left and then exposed to

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growth hormone or prolactin for 12 hours. Cell lysates were then prepared and CAT or luciferase enzyme activity measured. Both growth hormone and prolactin treatment lead to an approximately 5-fold stimulation reporter enzyme expression relative to transfected but non-hormone treated cells. These results show that both growth hormone and prolactin can regulate the reporter construct and that a requisite for this is the presence of SPI elements. The core element in the SPI-TK-reporter gene that confers GH regulation is likely to be, TTCTGAGAA, and similar results can be obtained with this element termed SPI-GLE as with the longer, 50 52 bp element named SPI-GHRE.

Replace the paragraph beginning at page 3, line 38, with the following amended paragraph:

Reporters plasmids containing one to six copies of the 50 52 bp SPI element fused to the TK promoter were constructed. The growth hormone responsiveness of these constructs was tested by transfection into a CHO cell line that stably expresses the rat growth hormone receptor DNA. Growth hormone stimulation of these cells showed that multimerization of SPI elements resulted in a larger growth hormone response.